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ISSN: 2278-1862

*Journal of Applicable Chemistry*

2019, 8 (6): 2386-2392

(International Peer Reviewed Journal)

# Isolation of new Volatile ester and Ellagitannin from *Glossocardia bosvallia* (L.f) DC-Asteraceae

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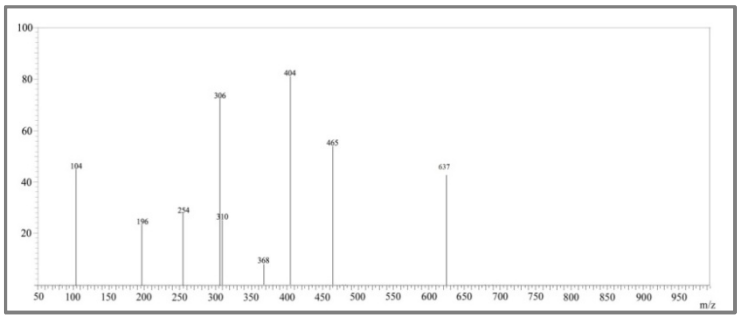
**Accepted on 3rd November, 2019**

## ABSTRACT

*The chemical exploration of whole plant methanol extract of Glossocardia bosvallia (L.f) DC-Asteraceae, an endemic medicinal plant from Western Ghats, led to the isolation and identification of two new compounds Veleric acid (volatile ester) and Corilagin (ellagitannin). The chemical structure of these compounds were elucidated and conformed by chromatographic and NMR Spectroscopic analysis. Earlier compound Veleric acid is the flavor components of the majority of fruits and the later Corilag in is a class of hydrolyzable tannins, a type of polyphenol responsible for many antioxidant properties. The isolated compounds were reported first time in the study plant G. bosvallia and may be expedient for several pharmacological activities.*

## Graphical Abstract




Mass Spectrum of isolated compound Corilagin from *G. bosvallia.*

**Keywords:** *Glossocardia bosvallia,* Veleric acid, Corilagin and NMR Spectroscopy.

## INTRODUCTION

Medicinal plants possess various therapeutic properties owing to the presence of various phyto-constituents present in them. These chemical constituents are categorized into primary and secondary

metabolites. They also occupy various parts of the plant leaves, stem, roots and fruits. But herbaceous plant possesses this type of chemical substance very minimum quantity and possibly by the parts above the soil (stems and leaves). *Glossocardia bosvallia* (L.f) DC belongs to the largest plant family Asteraceae. It is a small annual herb, with height of 10-20 cm and branched from the base; the branches are grooved. Leaves are alternate, and yellow flowers are borne in heads; stalks slender, axils or at the end of branches.

Traditionally the plant decoction was used by tribal inhabitants of Western Maharashtra as febrifuge and afresh paste is applied to promote healing of sores and wounds. It has a bitter taste and fennel like odor. This plant also used in culinary purposes apart from therapeutics [**1-3**].

## MATERIALS AND METHODS

**Collection of plant materials:** A healthy plant species of *G. bosavallia* was collected from the Western Ghats of Coimbatore district part of Madukarai hills. Voucher specimen (No. BSI/SRC/5/23/2014-15/Tech./1532) was deposited in Botanical Survey of India, Southern Regional Centre and Kongunadu Arts and Science College (Autonomous) [Coimbatore, Tamilnadu (India)].

**Preparation of extracts:** The freshly collected plants were washed in running tap water and shade dried for 15 days. After that the plant was pulverized into fine powder using pestle and mortar. Fine powder was packed with No.1 Whatman filter paper and placed in soxhlet apparatus along with solvents, petroleum ether; chloroform followed by methanol and aqueous extract was also taken. The extracts were collected and dried at room temperature, 30°C. The extract yielded was weighed and then stored for further usage.

**Purification of extract using column chromatography:** The dried powder was extracted in 200 mL Methanol. The extract was then passed through anhydrous sodium sulphate for the removal of aqueous matter. The material was then subjected to column chromatography after defeating. About 500 g of silica gel (Column Chromatography Grade-Mesh size 60-120) was kept in oven for one hour at 105°C for activation. It was cooled in desiccators for 1 hour. The methanol extract was then mixed well and dried by continuous stirring with activated silica gel of mesh size 60-120. A column of length 60 cm and 1.5 cm diameter was used for doing column chromatography. The column was packed up to 2/3 portions with the previously activated silica gel G (E.Merck) by wet packing procedure. The uppermost part of the column was packed with the dried extract mixed with the silica gel. Then it was covered by cotton so as to avoid the disturbance while pouring the solvent.

The solvent system used for the elution of the column is as follows (a) Each proportion was of 100 mL each, (b) 20 x 5 and (c) Total 4 combinations of Toluene: Ethyl acetate (8:2), Toluene: Ethyl acetate (6:4), Toluene: Ethyl acetate (4:6) and Toluene: Ethyl acetate (2:8). Similar fractions were clubbed together after analyzing with TLC. The column eluted fractions were washed and purified using DMSO/methanol.

**UV and IR Spectroscopy:** UV/Vis molecular absorption is used for the analysis of a diverse array of industrial samples including pharmaceuticals, food, paint, glass, and metals. Many pharmaceutical compounds contain chromophores that make them suitable for analysis by UV/Vis absorption. Products that have been analyzed in this fashion include antibiotics, hormones, vitamins, and analgesics. UV/Vis molecular absorption is routinely used for the analysis of narcotics and for drug testing.

The filter photometer is a simplest instrument for IR absorption spectroscopy. Typically are used as dedicated analyzers for gases such as HCN and CO. Infrared instruments using a monochromator for wavelength selection use double-beam optics easier to correct for the absorption of infrared radiation by atmospheric $CO_2$ and $H_2O$ vapor when using double-beam optics. Infrared spectroscopy is

routinely used to analyze gas, liquid, and solid samples. Sample cells are made from materials, such as NaCl and KBr that are transparent to infrared radiation [4].

**High Performance Liquid Chromatography Specifications (HPLC):** Liquid chromatography column having reverse phase (C-18-Aminopack Zorbax Eclipse-AAA) was used to pump at the speed of 10 avp. 10 µL of the filtered sample was then injected to the automatic injector using a Micro syringe (1-20 µL, Shimadzu). Mobile phase of methanol: water (90:10, v/v) at composition of 9:1 in an isocratic mode. The column used was RPC-18 (phenomenex). The flow rate was maintained at 1.3 mL $min^{-1}$ with a column temperature of 25±2°C. The class VP integration software was used for the data analysis. After purification, the isolated compound was once again subjected to HPLC with same specification to confirm the data of the compound after purification.

**Structural elucidation using Nuclear Magnetic Resonance (NMR) Spectroscopy:** NMR studies were carried out in the UWIN Life sciences, Malappuram, Kerala. The Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful method used in the determination of unknown organic compounds. Column purified fraction was dissolved in Dimethyl sulfoxide (DMSO) and analyzed by proton NMR ($^{1}H_1$) and carbon NMR ($^{13}C$). $^{1}H_1$ and $^{13}C$ NMR spectra were recorded in a Bruker DRX-500 MHz spectrometer (400.23 MHz for $^{1}H_1$ and 116. 7890 MHz for $^{13}C$), equipped with an Indy Silicon Graphics computer. Free induction decay (FID) was transformed with LB of 0.116 Hz and 1.0 Hz for proton and carbon NMR respectively.

## RESULTS AND DISCUSSION

The isolation of natural organic compounds especially from plants is very difficult because to obtain purity. Several concerns and methods are involved for the process one such analytical are spectroscopy methods like UV, IR, MASS and NMR. The present study is one such a kind of analysis which leads to isolation and identification of organic compounds from *Glossocardiabosvallia.*

The isolation and structural elucidation of a new allelo chemical 5, 6,7, 4′, tetrahydroxy-3-methoxyflavone-7-*O*-b-D-xylopyranosyl-(1→4)-O-b-D- glucopyranoside which showed antiviral activity, along with two known compounds 6, 4′-dimethoxy-5, 7-dihydroxy-flavone and Isoorientin was isolated by Yadava and Shirin [5] from methanolic extract of the stems of *G. bosvallia.* One of the compounds gave positive result to Molisch and Shinoda test showing its flavonoidal glycosidic nature [6, 7] (Table 1 and Figures 1-4)

**Table 1.** HPLC profiling of isolated compound Corilagin from *G. bosvallia*

| Peak Number | Retention Time in Min | Peak Area Obtained under the curve |
|---|---|---|
| 1 | 19.59 | 1002 |

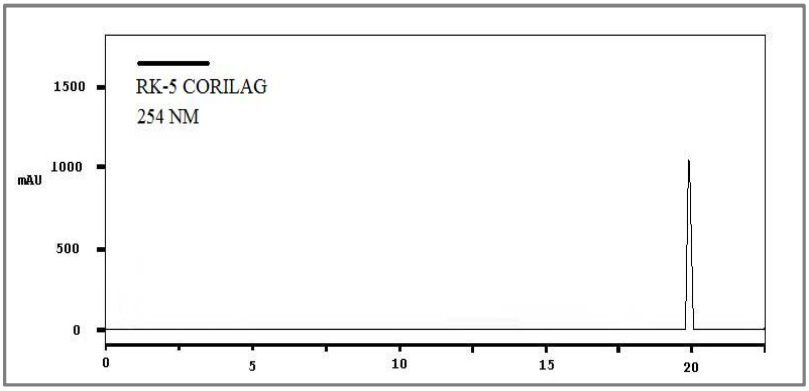



**Figure 1.** HPLC chromatogram of isolated compound Corilagin from *G. bosvallia*

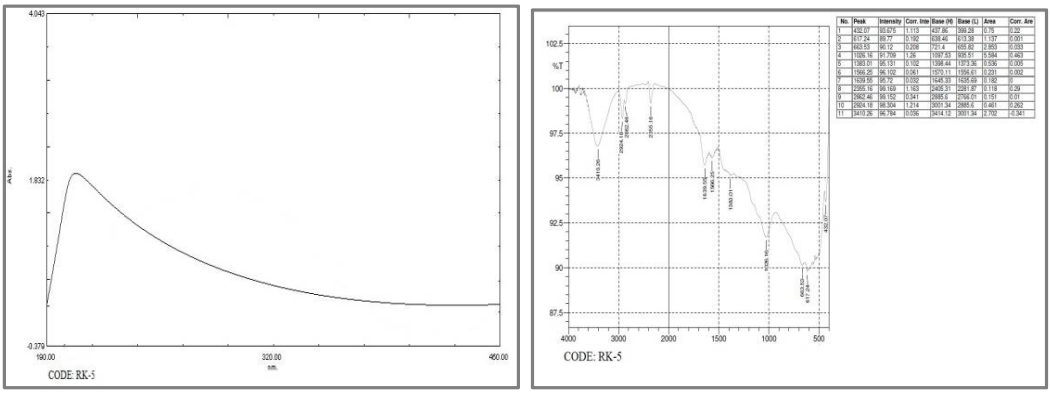



**Figure 2.** UV Spectrum of isolated compound Corilagin from *G. bosvallia.*

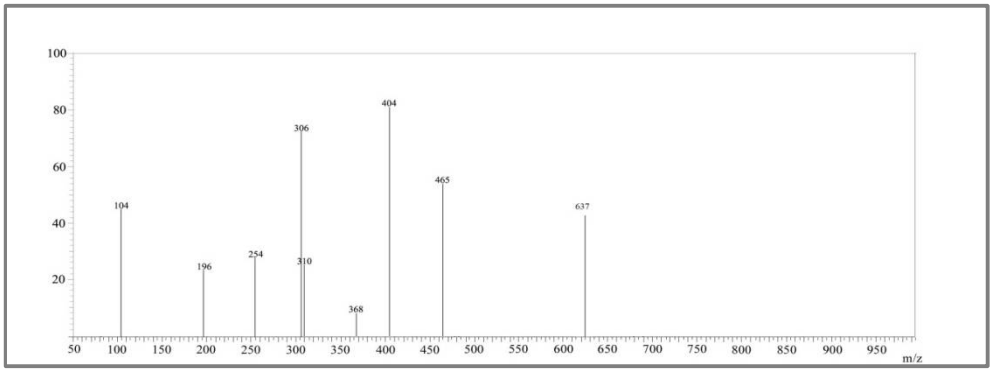



**Figure 3.** Mass Spectrum of isolated compound Corilagin from *G. bosvallia..*

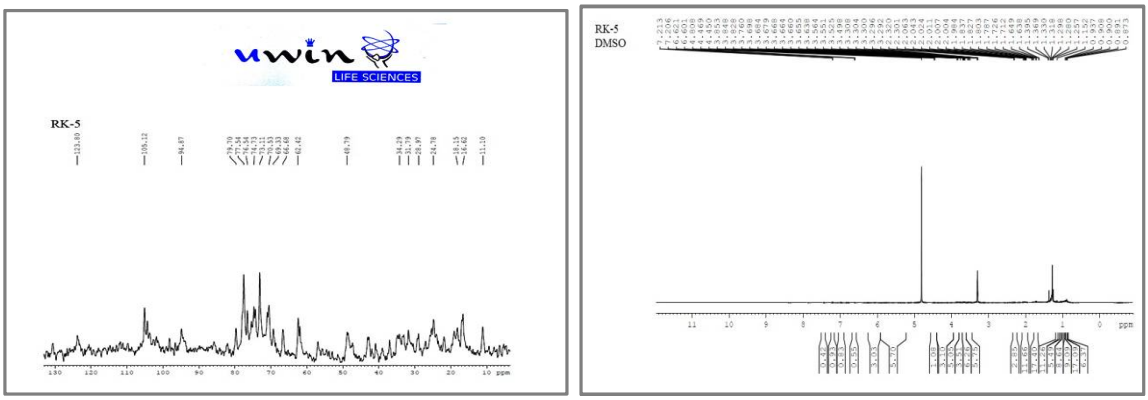



**Figure 4.** Carbon and Proton NMR Spectrum of isolated compound Corilagin from *G. bosvallia.*

The isolated compound also responded to neutral ferric chloride test. The UV spectrum showed absorption band which were indicating it to be flavone. Its IR spectrum showed strong absorptions to methanol confirmed the presence of -OH groups at C-5 and C-6 position of isolated compound from of *G. bosvallia* [**8, 9**] (Table 2 and Figures 5-8)

**Table 2.** HPLC profiling of isolated compound Valerenic acid from*G. Bosvallia*

| Peak Number | Retention Time in Min | Peak Area Obtained under the curve |
|---|---|---|
| 1 | 9.95 | 865 |

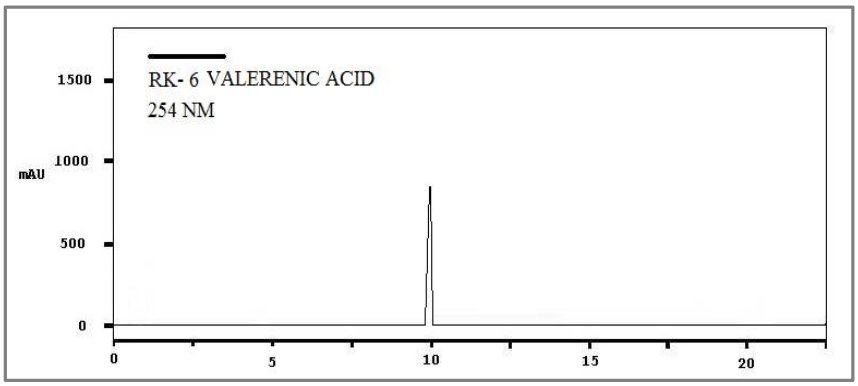



**Figure 5.**. HPLC chromatogram of isolated compound Valerenic Acid from *G. bosvallia.*

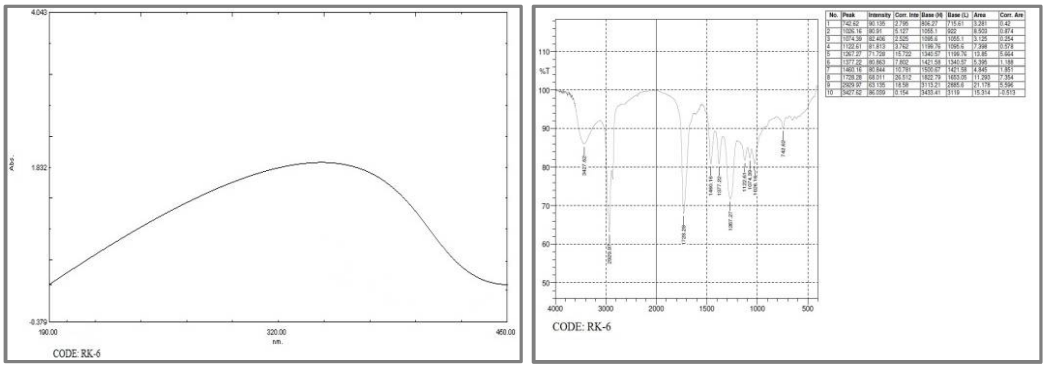



**Figure 6.** UV and IR Spectrum of isolated compound Valerenic acid from *G. bosvallia.*

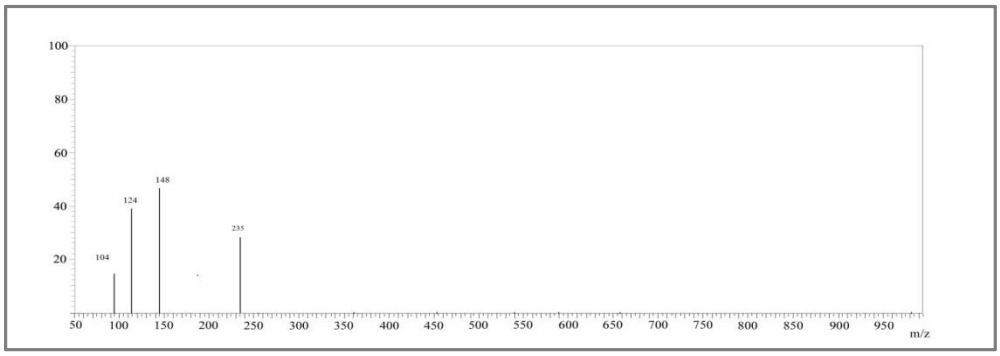



**Figure 7.** Mass Spectrum of isolated compound Valernic acid from *G. bosvallia*..

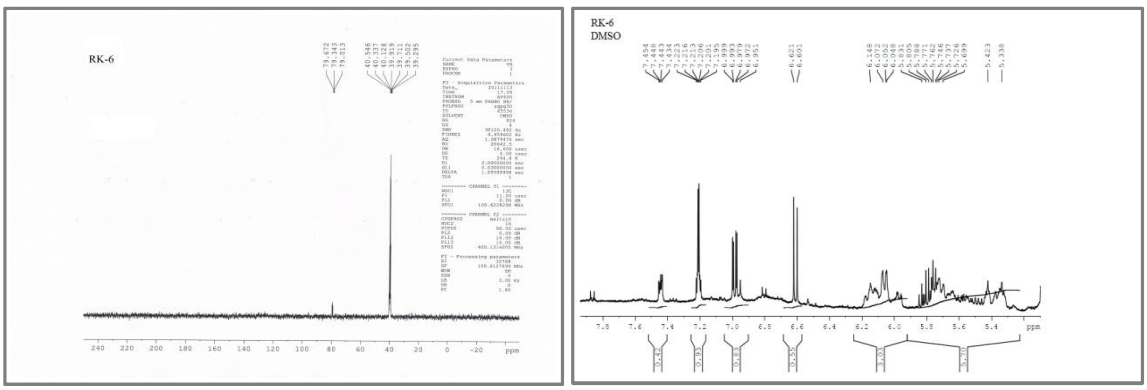



**Figure 8.** Carbon and Proton NMR Spectrum of isolated compound Valerenic acid from *G. bosvallia*..

## APPLICATION

In the present study the isolated and identified compound valerenic acid is a sesquiterpenoid and constituent of the essential aromatic oil from the plant of G. *bosvallia*. It is mostly used as herbal medicine for sedative, which may be helpful in the treatment of insomnia and analgesic properties [**10, 11**] reported that the valerenic acid acts as subtype-selective GABAA receptor positive allosteric modulator via a binding site in the trans membrane domain at the β+α− interface and receptors expressed in Xenopus oocytes (frog eggs) it was shown β2 or β3 subunits were stimulated [**12,13**] found the valerenic acid to inhibit NF-κB, protein complex that controls the transcription of DNA, in HeLa (cultured human cancer) cells line studies and possess anti-inflammatory properties. The other compound, Corilagin was isolated and identified from *G. bosvallia* which is derived from the ellagitannin classified under category of Tannins. Ellagic acid and corilagin inhibit TGF-β1-dependent EMT and has been shown to attenuate fibrogenesis in a mouse model [**14**] and Fibrosis is also indicated in many health conditions, including skin aging and MRSA susceptibility.

## CONCLUSION

In this study the two new compounds, Valerenic acid and Corilagin were isolated and identified from *Glossocardia bosvallia* and these compounds possess some promising importance as phytoconstituents or as volatile constituents in nature. Various biological studies already indicated the mechanism and nature of potential action of the compounds. Hence, the future research may lead for further exploration and findings of more biological importance from the study plant.

## ACKNOWLEDGMENT

The author K. R. Gopinath wishes to thank Dr. Samidurai, Post Doctoral Fellow, Department of Botany, Bharathiar University, Coimbatore and Dr. M. Saratha, Assistant Professor, Department of Botany, Nirmala College for Women Coimbatore for their guidance & encouragement. The authors also convey his gratitude to Dr.K. J. Rajendra Prasad, HOD Chemistry, Bharathiar University, Coimbatore for his continuous support throughout Research.

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